

CHAIN FORMING DIATOMS :  
EVIDENCE OF PARALLEL EVOLUTION  
IN *CHAETOCEROS*

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ABSTRACT. — The chains of the diatom genus *Chaetoceros* are an important part of the marine phytoplankton in many parts of the world ocean. The chains frequently have specialized end valves with the siliceous setae of the sibling intercalary valves permanently fused throughout the chain. *Chaetoceros radicans* Schütt from the southern Indian Ocean is an example of such chain formation, although the bifurcating spines on the setae give the chain an unusually forbidding appearance. The species has a labiate-like process on specialized end valves only and no chloroplasts in the setae, as is typical of the subgenus *Hyalochaete* Gran. It is bilaterally symmetrical and produces paired resting spores with specialized setate valves attached, as do some other coastal species. *Chaetoceros flexuosum* Mangin, in culture from SW Atlantic waters, contrasts in that the chains are held together only by the elaborate intertwining of the setae. Lacking bilateral symmetry, it has a labiate-like process on every valve and chloroplasts intruding into the setae, as is typical of the subgenus *Phaeoceros*. Another species that forms chains in a unique manner is *C. convolutum* Castracane, also in the subgenus *Phaeoceros*, studied here from both the southern Indian Ocean and the CEPEX site at Saanich Inlet, British Columbia. Lacking bilateral symmetry, it has a labiate-like process on every valve and heterovalvate cells — one valve rounded in girdle view and the other rectangular. Each seta from a rounded valve possesses a «prehensor», apparently adapted from a spine, that firmly grasps the seta from its sibling valve. These markedly different methods of chain formation within a single genus illustrate both primitive and advanced characters. Assuming the two last-mentioned species developed from single cells and not colony-formers, they also illustrate parallel evolution of the advantageous character of chain formation.

ABSTRAKT. — Die Ketten der Diatomeengattung *Chaetoceros* sind ein wichtiger Teil des Meeres-phytoplankton in vielen Teilen der Ozeane. Diese Ketten häufig besitzen spezialisierten Endschalen mit Kieselsborsten, die mit interkalaren Schalen befestigt sind. Ein Beispiel für solche Kettenbildungen ist *Chaetoceros radicans* Schütt vom südlichen indischen Ozean, obwohl die zweizästigen Dornen der Borsten eine besonders abschreckende Erscheinung

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nung erzeugen. Im Vergleich zur Untergattung *Hyalochaete* Gran besitzt diese Art nur an den spezialisierten Endschalen eine Fortsatz (ähnlich wie eine Lippenfortsatz) und keinen Chloroplasten in den Borsten. Diese Art sowie andere Küstenarten sind zweiseitig symmetrisch und erzeugen gepaarte Ruhe sporen mit Borstenschalen. *Chaetoceros flexuosum* Mangin, kultiviert von südwestlichen atlantische Gebieten, unterscheiden sich insofern, dass die Ketten nur bei ausföhrlich gewobenen Borsten zusammengebunden sind. Sie besitzen einen Fortsatz (ähnlich wie einen Lippenfortsatz) bei jeder Schale, die zweiseitig Symmetrie fehlt, und Chloroplasten drängen sich in die Schale ein, wie typischerweise besitzt die Untergattung *Phaeoceros*. Eine andere einzigartige Kettenform, die untersucht wird ist *Chaetoceros convolutum* Castracane (auch Untergattung *Phaeoceros*) vom südlichen indischen Ozean und von der CEPEX Stelle am Saanlich Inlet, British Columbia. Sie besitzen Fortsätze (ähnlich wie Lippenfortsätze) auf jeder Schale, die zweiseitig Symmetrie fehlt, und heteroschatische Zellen, eine Schale im Zellengürtelansicht rund ist, die andere rechteckig. Jede Borste der runden Schale besitzt eine Art Klammer («prehensors»), scheinbar von einem Dorn eingerichtet, der die Borste der schwesterlichen Schale festhält. Die drei Kettenbildungsmethoden innerhalb eine Gattung stellen primitiv und gleichzeitig fortgeschrittene Eigenschaften dar. Nimmt man an, dass die zwei letzten Arten sich aus einzelnen Zellen und nicht aus Kolonisatoren entwickeln, dann dienen sie als Beispiele für parallele Entwicklungen nützlicher Eigenschaften der Kettenbildung.

## INTRODUCTION

Chain formation in marine diatoms is common, and the known methods of cell linkage are summarized in FRYXELL (1978a). Although the reproductive units are still single cells within the chain, the chain as a whole has many possible functions (FRYXELL and MILLER, 1978). Within the genus *Chaetoceros*, a few species are considered to exist only as single cells (e. g. HUSTEDT, 1930; LEBOUR, 1930), although most species are recorded as chain-formers in those references and in others (BRIGHTWELL, 1856, 1858; SCHUTT, 1895; KARSTEN, 1905; MANGIN, 1915, 1922; HUSTEDT in SCHMIDT, 1921; HUSTEDT, 1958; HENDEY, 1964). The type species of the genus *Chaetoceros* is *C. dictyota* Ehrenberg (1844), a chain-forming species described from antarctic waters.

Frustules of *Chaetoceros* are formed of the same units as are those of other diatoms, namely two valves connected by many open girdle bands. A valve is commonly ovoid, and each possesses two hollow, tube-like outgrowths (setae) projecting outside the valve margin and having a different, more open structure than the valve (Anonymous, 1975). Some characters for identifying species from field samples include size and shape of cells and of individual valves, presence or absence of a central labiate-like process on the face of the valve, clear constrictions on the girdle, number of chromatophotes, and resting spore morphology. Additional characters involve the setae: their configuration, whether they overlap inside or outside the diameter of the chain, the size and shape of the space thus left between cells, and whether or not there are specialized end valves of a chain with distinctive setae.

Many setae have spines. EVENSEN and HASLE (1975) found the setae may be round, square, or polygonal in cross section and are perforated in a definite grid-like pattern that is consistent within a single species. The usual

pattern of chain formation in the family Chaetoceraceae occurs by the fusion of setae from two sibling valves formed within a parent cell, with setae growing outside the diameter of the parent through rounded notches («setal indentations», Von STOSCH, 1975) in the last band of both the epitheca and the hypotheca of the parent cell (Von STOSCH, THEIL and KOWALLIK, 1973; EVENSEN and HASLE, 1975; see fig. 27 of *Bacteriastrium*, FRYXELL, 1978a). The setae have long been used for taxonomic groupings within the genus, with the subgenus *Phaeoceros* Gran (1897) including all the *Chaetoceros* species that have chromatophores penetrating the setae and the subgenus *Hyalochaete* Gran (1897) including those species possessing setae with no chromatophores.

Many species of *Chaetoceros* form resting spores (BLASCO, 1970, Von STOSCH, THEIL and KOWALLIK, 1973; HARGRAVES, 1976, 1979; HARGRAVES and FRENCH, 1975), which SIMONSEN (1979) considered an evolutionarily primitive trait and ROSS and SIMS (1974) considered typical of neritic species, at least in present day. HARGRAVES and FRENCH (in press) have recently reviewed diatom resting spore ecology. From the literature and from our own observations it appears that either one or two resting spores are formed from a *Chaetoceros* vegetative cell. These resting spores have distinctive morphology and are of taxonomic value, showing genetic divergence even when vegetative frustule morphology shows little if any divergence (HARGRAVES, 1979).

Of special interest in this paper is the pattern followed when two resting spores per vegetative cell are formed, often remaining joined. In this case the first division is equal, and if there are unusual setae on the sibling valves, it is apparent that resting spore formation has started. The next divisions of the nuclei are not synchronous, according to Von STOSCH, THEIL and KOWALLIK (1973), and the first arched resting spore valves are laid down as hypothecae to valves with unusual setae. The next unequal division in each cell results in a heavy hypovalve completing each resting spore, with the flattened, last-formed valves nested within the valves having unusual setae that hold the resting spore pairs together. When pairs do come apart, the valves with the setae are lost, as the empty vegetative thecae were earlier, and the spores separate (see fig. 53 and Discussion section). No rudimentary valves have yet been found in these empty thecae, although they have been noted in some other diatoms (DREBES, 1966; SYVERTSEN, 1979; HOBAN, FRYXELL and BUCK, 1980; FRYXELL, DOUCETTE and HUBBARD, 1981). Von STOSCH and others (1973, 1979) call this an example of acytokinetic mitosis (= nuclear division without cytokinesis, DREBES, 1970), since no residual body or rudimentary valve has been found. Apparently mitosis is essential for valve formation, as has been pointed out by Von STOSCH and KOWALLIK (1969), but in different species cytokinesis is more or less unequal in the determinate divisions leading to resting spores formation. Von STOSCH and FECHER (1979) consider unequal mitosis with little loss of cellular material to be an advanced characteristic.

Markedly different methods of chain formation have been found in three species of *Chaetoceros*, resting spores being noted in only one case.

## MATERIALS AND METHODS

Cultures and preserved samples were studied in light and electron microscopes. They were either studied in water mounts to observe whole cells and chain formation or rinsed or cleaned of organic matter (SIMONSEN, 1974; MA and JEFFREY, 1978) and mounted for viewing in the scanning electron microscope (SEM), Jeolco JSM-35, Electron Microscopy Center, TAMU, or permanently mounted for light microscopy (LM), with micrographs taken on the Zeiss inverted microscope, ICM-405, using phase or differential interference contrast.

Cultures of *Chaetoceros flexuosum* were obtained from the cooperative Argentinian Cruise ISLAS ORCADAS 19/79 in the southern Atlantic Ocean, Station 12 (64°04.8'S, 48°42.2'W, 16 March 1979). A raw water sample was returned on ice by R.A. Warner to Texas A & M University where twelve clonal cultures are now growing in 4°C growth chambers in f/2 medium: AA22, isolated by S.M. Schauffler, AA34-AA43 and AA45, isolated by senior author and used for this study.

*Chaetoceros radicans* was collected by D.A. Stockwell under the direction of S. Z. El-Sayed from the southern Indian Ocean, on the cooperative French Cruise MARION-DUFRESNE MD08. In this study, concentrated samples were used with micrographs from three stations: St. 5, 27°43.6'S, 46°26.0'E, 13 Mar. 1976, 300-0m net haul; St. 17, 46°52.6'S, 37°53.7'E, 25 Mar. 1976, 100-0m net haul; St. 27, 46°46.6'S, 37°52.4'E, 28 Mar. 1976, 150-0m net haul.

*Chaetoceros convolutum* was studied from the above cruise with micrographs from MD08, St. 5 (as above) and St. 49, 46°06.0'S, 50°34.5'E, 16 April 1978. Figures are also included from net samples taken at the CBPEX site, Saanich Inlet, British Columbia, 48°39.6'N, 123°29.06'W, 8 July 1978, collected by the senior author and providing examples from a northern population.

Terminology is that recommended by the Working Party on Diatom Terminology, Third Symposium on Recent and Fossil Marine Diatoms, Kiel, 1974 (Anonymous, 1975) and Von STOSCH (1975). Following HENDEY (1964) the word *Chaetoceros* is treated as a Latin neuter noun with the specific epithets in agreement.

## RESULTS

### *Chaetoceros flexuosum* Mangin

MANGIN (1915, p. 45, fig. 27, Pl. 1, fig. 7) described this distinctive species from antarctic waters, stressing the twisted appearance of the setae (fig. 50). Sibling setae are flexed and cross each other to form one or two turns. MANGIN stated that the chains are compact with the cells closely appressed. Terminal setae are regularly curved into arcs, sometimes with a double curve.

In our material, rapidly growing cells were crowded with chromatophores that extended into the setae (fig. 1-3). Setae diagonally across the cell are in focus at one time (fig. 1) with one diagonal pair originally curving to the front of the cell and the other curving back. Senescent cultures had single cells only in culture material, but no resting spores. Chains of two, three, and more cells (fig. 2, 3, 5) were common in rapidly growing cultures. Although occasionally the polarity of the chain appeared to change in the middle of chain, the setae usually tended in the same direction, intertwining as MANGIN had stated (fig. 4).

In SEM, the asymmetry of the cell is confirmed (fig. 16, 19) and includes a labiate-like process that is located toward one margin from the center of the valve (fig. 17, 21) and seen from the inside as a simple hole (fig. 22). If we consider the labiate side as «ventral», the right seta goes in front of the sibling seta and the left goes behind it on all valves studied thus far. Sibling valves, whole cells, and apparently chains had one ventral side. The valve is delicately structured and perforated (fig. 21, 22). The perforated girdle bands are open with pointed ends that fit together (fig. 18, 20).

Sibling valves are held together by intertwining setae, and not by the fusion of silica, as is more common in *Chaetoceros*. SEM clarifies what has been noted in LM. In «ventral» view, the upper valve has slipped to its left in fig. 23a, 23b, and to its right in fig. 25, illustrating that sibling valves are not held firmly in place. In fig. 24a, the «dorsal» view shows the top valve has slipped to its left, and details of each side (fig. 24b, 24c) show that the setae are intertwined loosely without any evidence of fusion, not has any been noted in this study. The setae have spines spiralling along thickened ribs (fig. 27) and are thicker near the valve and much thinner distally (fig. 28).

MANGIN (1915) found this species to be rare or very rare from 65°-70°S along the coast of Antarctica in the SE Pacific from the POURQUOI-PAS Cruise. It has not been reported often in the literature, but it is not uncommon. MANGUIN (1960) considered it rare or very rare in seven stations in the Antarctic Zone (= the region between Antarctica and the Antarctic Convergence Zone) south of Tasmania on the COMMANDANT-CHARCOT Cruise. HASLE (1969) classified it from the BRATEGG Cruise as a diatom species with its northern limit within the Antarctic Zone. The senior author has found it from ELTANIN Cruise 46, southern Indian Ocean, from 64°23'S, 114°26'E, 11-12 Dec. 1970, and 64°32'S, 104°42'E, 15 Dec. 1970. It was also present in five out of 11 net hauls examined from ELTANIN Cruise 51 in the Ross Sea area from 71°50.6'S, 179°29.4'W, 6 Feb. 1972, to 77°10.4'S, 172°04.3'W, 22 Feb. 1972. K.R. BUCK (1978) has found it in the Weddell Sea at 65°23.0'S, 51°28.0'W, 19 Feb. 1977, and at all 11 stations between 71°25.0'S, 45°00.0'W, 24 Feb. 1977, and 77°46.0'S, 35°10.0'W, 28 Feb. 1977, from collections on the USCG BURTON ISLAND. It was isolated from the southern Atlantic Ocean and cultures (see Materials and Methods section) for the present study, providing abundant material. It is a truly planktonic, antarctic species.

*Chaetoceros radicans* Schütt

In the original description SCHÜTT (1895, p. 48, fig. 27) was very brief but stressed the branched spines in the «lower part» (distal part) of the setae. His drawing is reproduced in fig. 51, from a paper that described 26 new species and one new variety in the genus *Chaetoceros*, plus a new related genus. Although the description of this species is the shortest in the paper and the drawing shows few characters, the structure of the setae is generally considered diagnostic.

The chains were long and curved in our preserved material (fig. 9, 10) with the spiny setae not all in the same plane. In valve view, a sibling pair of vegetative valves show the setae configuration (fig. 11). There is no process in the center of the intercalary valves (fig. 11), but a labiate-like process is visible in LM in the center of the differentiated end valve (fig. 12). In the SEM, the degree of branching on the setae of sibling intercalary valves is clear (fig. 29, 30). As previously noted in Chaetoceraceae (FRYXELL, 1978a), the setae of sibling valves are separate (fig. 32), although fused at a sharp angle in this case holding the valves apart (fig. 31). The bifurcating tube-like spines develop fully beyond the fused area and diminish as the setae taper to thin threads. One need only imagine how effectively such structures clog filtering devices of grazers. The end valves (inside view, fig. 33; outside view, fig. 35) are differentiated by the presence of a labiate-like process, lacking in the intercalary sibling pairs (fig. 34) as noted above.

The resting spores are formed within vegetative cells in pairs (fig. 13) and cling together after the vegetative thecae are lost (to be more fully discussed in the next section). In valve view (LM) the resting spore pairs are distinctive with recurved, crossing setae that lack long bifurcating spines (fig. 14). In SEM the structures become clearer. The first-formed specialized valves with crossing setae are not integral parts of the resting spores (fig. 36), although they remain part of the complete resting spore pair (fig. 37, 41) and function as epithecae with girdle bands of the first-formed, arched resting spore valve (fig. 39a, 39b). The setae do have spiraling, dendritic spines (fig. 39a), although they are usually dwarfed (fig. 38). The single resting spore (fig. 40, 42) thus has a highly arched epitheca formed with little resistance from a sibling cell in an empty (or nearly so) vegetative theca plus a flattened hypotheca that formed against the sibling valve with the specialized, recurved setae. The spores themselves have no girdle bands or setal openings into the cell (although rudimentary knobs have been seen), and yet they have what protection setae can offer. If the specialized setae are broken or if pressure is put on them, the spores can be released singly. (See next section and fig. 53).

The type location of the species is given simply as «Atlantic». HUSTEDT (1930) reports it from coastal areas of Europe and the Mediterranean Sea. SIMONSEN (1974) found it to be rare in his samples from the Arabian Sea with more regular occurrences on the transects off India in the Indian Ocean from samples collected on the METEOR. It was never frequent, but he considered it to be cosmopolitan. It was frequently encountered farther south in the

Indian Ocean from the MARION DUFRESNE MD08 collections, in 19 of 39 stations, with net hauls dominated by this species NE of Marion Island in six stations (FRYXELL, 1978b). Although it has not been found in the polar areas, it has a wide distribution; following HASLE's (1976) usage, SIMONSEN's term «cosmopolitan» certainly applies to this species.

### *Chaetoceros convolutum* Castracane

CASTRACANE (1886, p. 78) described this species from a surface collection made between Kerguelen and Heard Island in the southern Indian Ocean. He stressed that the cells were heterovalvate with the one valve being convex and the other flat. The very long setae originate toward the center of the valve and are convolute, curving around each other (fig. 52). The end valves of the chain are not shown, although he refers to them as embryonic, short protuberances (probably broken).

Rounded end valves are shown in fig. 6, 8, with a whole cell from the same («upper») end in fig. 7. The other terminus is not shown. The setae do arise in the middle of the valve, and the large setae are spiny. In SEM, the rounded terminal valve has a marked constriction near the margin (fig. 43c) also seen in LM, and the setae near the valve have many spines (fig. 43b). Away from the valve the setae get slightly larger, while the spines get longer and less frequent (fig. 43a). A rounded intercalary valve shows some of the same characteristics (fig. 44a) with a labiate-type process nestled under the curve of the setae (fig. 44b). A sibling pair of valves is heterovalvate, with one being rounded and the other more rectangular (fig. 45a). The rounded valve has a flap or prehensor on each seta that holds the sibling seta in place, shown in detail in fig. 45b. Broken prehensors can also be seen in fig. 44a, curving back toward the rounded valve with no evidence that they were fused to the rectangular valve. Prehensors are lacking in rounded end valves. This method of linking sibling valves has not previously been reported in *Chaetoceros* (possibly present in *C. denticulatum* Lauder?). Figures 43-45 are taken from the southern Indian Ocean.

Similar cells were found off Saanich Inlet, British Columbia, at the CEPEX site (see Materials and Methods section for location). A large heterovalvate cell of *Chaetoceros* was found (fig. 46a) with prehensors on the setae of the rounded valve (fig. 47, 48a). The prehensor shown in fig. 46a, 46b is partly broken or worn away, but the break shows no indication of fusion. The setae are spiny (fig. 46c), quadrangular in cross section, and with two rows of pores between circumferential ribs (fig. 48a, 46c). More distal parts of the setae have spines farther apart than at the proximal ends (fig. 48b). Each valve has a «ventral» side with a labiate-like process nestled between the setae (fig. 47, 49a, b), and its right seta curves in front of the sibling seta.

Identification problems will be discussed in the next section, but CASTRACANE's original collections were made from the southern Indian Ocean in what can be considered antarctic waters. In addition to the locations cited in a previous section, we have found it at MD08, St. 47, 46°12.9'S, 50°44.4'E, collec-

ted 15 April 1976. We have some doubtful citings in the Weddell Sea, also. Since the prehensor can be reliably noted only in SEM, we are hesitant to trust our LM observations until other features become clearer. If we are correct in assigning both the north and the south samples in our collection to *C. convolutum*, it is, indeed, a widespread species.

## DISCUSSION

### Resting Spore Production

*Chaetoceros radicans* follows the pattern of *C. didymum* Ehrenberg in resting spore formation. By adapting portions of von STOSCH, THEIL, and KOWALLIK's (1973, fig. 26) information-packed schema of the life cycle of *C. didymum*, we can clarify points made in the Introduction and features noted on micrographs in the Results section. In fig. 55, a vegetative cell is beginning division in A, producing two equal cells with hypothecae possessing specialized setae (SS, fig. 55B). In fig. 55C the cells have elongated, and acytokinetic division has taken place in the upper cell, with one arched resting spore valve (AR) formed as hypotheca to the valve with specialized setae. In fig. 55D the lower cell has the arched resting spore valve, and the upper cell has undergone another acytokinetic division and formed a flattened resting spore valve (FR) as hypotheca to the arched valve. The vegetative epitheca (VE) has been lost, as an empty (or nearly empty) husk. In fig. 55E, the lower cell has gone through the same sequence, and the doublet resting spores are complete. Figure 55F shows the resting spores (RS) separated from the first-formed valves with the specialized setae, a process that may or may not occur before germination.

Thus, for each two resting spores formed one vegetative cell, the original two valves and bands are lost, plus two valves with specialized setae and their bands. There is no evidence at this time that cytoplasm is lost - that is, no residual bodies have been noted in the empty thecae. In fact, von STOSCH, THEIL and KOWALLIK (1973) show the pycnotic nuclei as a part of the surviving cell of *C. didymum*. And we have seen no evidence that rudimentary valves have been formed during the unequal mitotic divisions, as has been found in *Thalassiosira* (FRYXELL, DOUCETTE, and HUBBARD, 1981) or *Eunotia* (Von STOSCH and FECHER, 1979). However, the resting spores are produced by a determinate number of divisions as in other diatoms, with what seem to be minor variations. However, in no other genus thus far studied is the first specialized valve produced not an integral part of the resting spore.

### Identification Problems

The classical features used in field identification of *Chaetoceros* species were summarized in the introduction, and this work has stressed the nature of the setae in three unusual cases. In some cases, however, there are problems in determining which species is being studied.



Apparently there are no problems with *C. flexuosum* (fig. 50). It is confined geographically to the truly antarctic waters, and the records of its occurrences (by admittedly few authors) show that it is widespread in a band around Antarctica. The morphology observed by the different authors is consistent.

There may be problems with *C. radicans* (Fig. 51) as with any cosmopolitan species that is identified principally by one character - in this case, by the elaborate setae with bifurcating spines. Two published accounts with drawings of the resting spores (LEBOUR, 1930, p. 163, as *C. scolopendra* Cleve; HENDEY, 1964, p. 134, pl. 14, fig. 3) are not completely consistent with the micrographs and descriptions in this paper. HENDEY and also LEBOUR mention peculiar thick setae of the spores encircling the girdle, but the drawings show no setae between the flattened valves of the resting spore pairs. In truth, the resting spores were not mentioned in SCHÜTT's original description, and the possibility remains that resting spores in this genus may show early speciation before the vegetative phases do (HARGRAVES, 1979). Thus, they may well show variation from one geographical region to another, although the disagreement in the verbal account and the drawing leaves some doubt in this case. The *C. radicans* (= *C. scolopendra* Cleve?) of the North Sea, the English Channel, and North Atlantic coasts deserve particular study. HENDEY's (1964, pl. 14, fig. 4) figure of the vegetative cells agrees well with our observations.

Identification problems are most evident in *C. convolutum*. CASTRACANE described this species (fig. 52) at the same time as he described *C. criophilum*. The difference between this and a northern species was not readily apparent to many diatom workers, and MANGIN (1917a) published clarification of *C. criophilum* (fig. 53) pointing out that it is an antarctic species. The same year, he also published the description of a new species, *Chaetoceros concavicornis* (MANGIN, 1917b) that he considered to be an arctic form (fig. 54) in contrast to *C. criophilum*. He was surely dealing with two different taxa, and his careful work is helpful. He also dealt directly with the differences between *C. concavicornis* and *C. convolutum* (MANGIN, 1919), citing the existence of a large band on *C. convolutum* and a narrow or indistinct one on *C. concavicornis*. HOLMES (1956) pointed out the problems related to this character on solitary cells in water mounts and dividing cells. With the present observations of *C. convolutum* from the southern Indian Ocean and the NE Pacific, new problems are raised, and comparisons are necessary for critical work.

The heterovalvate species that have one rounded and one rectangular valve include: *C. criophilum*, *C. pendulum* Karsten, *C. peruvianum* Brightwell, *C. concavicornis*, and *C. convolutum*. *C. criophilum* and *C. pendulum* are set apart in that the setae arise near the edge of the valve. They have different outlines with the upper valve being more rounded in *C. criophilum* and the setae more spiny.

*Chaetoceros peruvianum* is most often shown as a single cell, with some space showing between the central arch of the joined setae on the rounded («upper») valve, as shown by BRIGHTWELL (1858). HUSTEDT (1930, fig. 379) did show three loosely connected cells, but they could hardly have been

permanently fused sibling cells in a chain, since such fusion takes place within the girdle bands of the parent cell, and the cells in the figure are much more widely spaced than that. Wide spacing between cells in a chain-forming species is considered advanced (BEKLEMISHEV, 1961), and it could well be that *C. convolutum* with its prehensor evolved from a chain-former with valves more closely appressed or a single-celled form. Most *Chaetoceros* species have only single cells under unfavorable growth conditions (e. g. senescent cultures) in the TAMU Culture Collection. The chains have not come apart, but each cell has made two specialized end valves when dividing. It would be interesting to see if *C. peruvianum* could form chains in culture under ideal conditions and what kind of chains they would be.

A problem remains in identification of *C. concavicornis* and *C. convolutum*. A culture of *C. concavicornis* kindly sent from D.G. Swift (SWIFT and GUIL-LARD, 1978) was examined (also studied in EVENSEN and HASLE, 1975), and no prehensor is present. Sibling valves are fused. Two taxa are involved, at least. It may be that the species HUSTEDT (1930, fig. 378) illustrated as *C. convolutum* is a third taxon since the setae arise in the middle of the cell only on specialized end valves, and that is not consistent with present observations, as previously noted. However, BRAARUD (1935) showed variation in this character within one chain (perhaps in part due to the torsion of the chain) and suggested the reduction of *C. concavicornis* to a form of *C. boreale* Bailey. EVENSEN and HASLE (1975) agreed that a few cells of a chain could resemble *C. boreale*, but whole chains could not be confused, with setae of *C. concavicornis* all pointing to the same end of the chain. Field characters have been used (drooping setae and three evenly spaced girdle sections on *C. convolutum*; CUPP, 1943, fig. 67; cf. *C. concavicornis*, fig. 66) that may have to be reevaluated in the light of new observations.

The situation is further complicated by finding of cells we consider to be *C. concavicornis* in the Weddell Sea. If we are correct, both *C. concavicornis* and *C. convolutum* are cosmopolitan or, more likely, bipolar in geographic distribution. It is as yet uncertain how many taxa are included in this heterovalvate group of *Chaetoceros* or what characters can be used reliably. The fine structure of the setae of *C. concavicornis*, *C. boreale* (EVENSEN and HASLE, 1975), and *C. convolutum* (present work) appear identical on field samples. Prehensors are distinct only in SEM.

## TAXONOMY

*C. radicans* has the manner of setae connection that is dominant in the genus and can be considered primitive in its chain formation. It is the only *Chaetoceros* species considered in this paper that possesses bilateral symmetry and resting spores. There are labiate-like structures only on end valves, indicating specialized chain-terminating cells. Within the genus *Chaetoceros*, it belongs to the subgenus *Hyalochaete* with no chloroplasts in the setae and section

*Furcellata* with paired resting spores that have thick bristles and relatively smooth valves.

*C. flexuosum* is placed in the subgenus *Phaeoceros* with chloroplasts not only in the cell but also in the setae, but it does not fit well into existing sections. The flexibility and small movements permitted by the setal arrangement of *C. flexuosum* may bestow some advantage on this species in an ever-moving water column. *C. flexuosum* appears to have the most simple manner of setae connection. However, since it is an isolated case of such intertwining in a large and heterogeneous genus where fusion is the rule, it would appear that *C. flexuosum* has lost the ability to fuse, rather than retaining the primitive condition. It may have developed from single cells. Unlike *C. radicans*, the cells are not bilaterally symmetrical, and there are labiate-like processes on all valves.

It is tempting to hypothesize the following sequence of events: *Hyalochaete* cells carried offshore into unfavorable growth conditions, surviving as single cells with both valves having specialized terminal structure from chains - both possessing labiate-like processes, utilizing larger, more open setae and chloroplasts in the setae as more effective means of exchange with low nutrient levels of oceanic seawater, and later the cells in general reverting to chain formation as the oceanic subgenus, *Phaeoceros*. If this sequence occurred, most species have again utilized fusion as a means of sibling valve adherence, but retained labiate-like processes on all the valves. A few species have remained as single cells, as *C. peruvianum*, and at least two other methods of chain formation have evolved, as illustrated by *C. flexuosum* and *C. convolutum*. The sequence, of course, could have been inverted, but if capability of producing resting spores is both coastal and primitive, the order of events outlined above has some merit. The fossil record may be more helpful in this genus if we concentrate on resting spores in the future.

*C. convolutum* is the most complex in its manner of chain formation, with the prehensors of one valve holding the sibling valve firmly in place. It lacks bilateral symmetry and possesses heterovalvate cells. There are labiate-like processes on all valves, as EVENSEN and HASLE (1975) found typical of the subgenus *Phaeoceros*. *C. convolutum* is placed in the section *Borealia*, although the type species of this section is usually considered isopole (GRAN, 1904, cited in BRAARUD, 1960). *C. convolutum* may also have developed from a single-celled form, in this case, perhaps a species such as *C. peruvianum*.

SIMONSEN (1979) holds that resting spore production is primitive, and it would appear that fusion of silica in chain formation is primitive in the genus *Chaetoceros* and that intertwining and prehensors are advanced. Thus within this one genus where setae are instrumental in chain formation, three different methods have been presented. One is dominant; two are isolated occurrences. And yet the function of chain formation is fulfilled - an indication of parallel evolution.

## ACKNOWLEDGEMENTS

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#### FIGURE LEGENDS

Fig. 1-8. — 1-5: *Chaetoceros flexuosum* Mangin, TAMU living culture AA22. LM. Scale: 10µm. 1: Single cell packed with chloroplasts extending into the setae. Note angle of lower setae. 2: Two cell chain showing closely appressed sibling valves. 3: Three cell chain with different perivalvar axes. 4: Detail of chain to illustrate manner of intertwining of setae. 5: Long, but incomplete chain with broken cell at end and cytoplasm plasmodized in death. 6-8: *Chaetoceros convolutum* Castracane, MD08, St. 49, LM, Scale: 10µm. 6: Rounded terminal valve of chain with drooping setae. 7: Whole terminal cell from broken chain. 8: Rounded terminal valve with flared setae.

Fig. 9-15. — *Chaetoceros radicans* Schütt, MD08 St. 17 except fig.13, LM. Scale: 10µm. 9: Chain with cells clearly separated and spiny setae. 10: Long, curved chain with terminal setae differentiated. 11: Valve view of sibling intercalary setae cleaned of organic matter with setae in typical configuration. 12: Valve view of terminal valve central labiate like process and terminal setae extending in different directions. 13: MD08 St. 27. Resting spore pair in vegetative cell with portion of seta in focus on right. 14: Valve view of resting spore pair with setae recurved and crossing in typical configuration (Corresponding to fig. 55E turned 90° to give valve view). 15: Resting spore pair, a: high focus on valve detail; b: mid-focus on valve outline and parts of setae (Scale for fig. 13-15 same as fig. 12).

Fig. 16-22. — *Chaetoceros flexuosum* Mangin, TAMU culture AA39, except fig. 18, 20. SEM. Scale: 1 $\mu$ m except fig. 16. 16: Chain of four cells in usual configuration. Scale: 10 $\mu$ m. 17: Valve view with labiate-like process on «ventral» side of valve, its right seta directed forward first and its left seta directed backwards slightly. 18: Antarctic rough culture from ISLAS ORCADAS, St. 12. Portion of girdle with perforate bands and pointed ends. 19: Whole end cell, somewhat collapsed. 20: Antarctic rough culture from ISLAS ORCADAS, St. 12. Portion of girdle with evenly perforate bands away from open ends. 21: Labiate-like process on outside of ventral mid-valve. 22: Oblique view of whole valve with the labiate-like process seen on the internal ventral side as a simple hole.

Fig. 23-28. — *Chaetoceros flexuosum* Mangin, TAMU culture AA39, except fig. 26. Sibling valves. SEM. Scale: 1 $\mu$ m except where noted. 23: «Ventral» view showing labiate-like processes, with upper valve having slipped to its left. a. Orientation view. Scale: 10 $\mu$ m; b. Detail of setae with no indication of fusion. 24: «Dorsal» view with labiate-like processes not showing, and upper valve again slipped to the left. a. Valves with setae. Scale: 10 $\mu$ m; b. Detail of setae twisted around each other with no fusion; c. Other pair of setae with space between them. 25: «Ventral» view with upper valve slipped to its right. No evidence of fusion. Scale: 10 $\mu$ m. 26: Rough culture, ISLAS ORCADAS 19/79, St. 12. Detail of twisted setae with spines but no fused areas. Note pores on mantle of valve and setae. 27: Detail of seta some distance from valve with spines on a thickened, spiraling rib. 28: Comparison of thick seta near valve and thinner part more distal from valve.

Fig. 29-35. — *Chaetoceros radicans* Schütt. MD08 St. 17, except where noted. Siliceous material cleaned of organic contents. SEM. Scale: 1 $\mu$ m except where noted. 29: MD08, St. 5. Single pair of sibling valves within chain; permanently bonded with spiny setae. Scale: 10 $\mu$ m. 30: Detail of same valves. Scale: 10 $\mu$ m. 31: Detail of setae fused between valves. 32: Pair of setae broken from valves, changing directions at point of fusion but still separate. 33: Inside view of specialized end valve in chain with slit. 34: Intercalary pair of valves with no labiate-like central process. Scale: 10 $\mu$ m. 35: Outside view of terminal valve of chain with slit of labiate-like process. Note delicate radial thickenings of valve.

Fig. 36-42. — *Chaetoceros radicans* Schütt. MD08, St. 17. Resting spores and attached valves. SEM. Scale: 1 $\mu$ m, except where noted. 36: First formed valves of resting spore sequence with specialized setae after spores have dehisced (corresponding to RS in fig. 55F). Scale: 10 $\mu$ m. 37: Resting spore pair still joined to valves with specialized recurved, crossing setae (corresponding to fig. 55E). Scale: 10 $\mu$ m. 38: Detail of specialized seta with spiraling rows of blunted spines. 39: Unusual case in which valves with specialized setae have broken apart. a. Orientation view; b. Rare outside valve view of first-formed valve of resting spore sequence, with multiple bands. 40: Single resting spore with arched valve (epivalve) and flattened valve (hypovalve) with no setae (corresponding to RS in fig. 55F). 41: Oblique view of resting spore pair still attached to valves with specialized recurved setae. 42: Girdle view of single resting spore with arched epivalve and flattened hypovalve (corresponding to fig. 55E, RS).

Fig. 43-45. — *Chaetoceros convolutum* Castracane MD08, St. 49, except where noted. Valve and seta structure, cleaned of organic material. Scale: 1 $\mu$ m, except where noted. 43: Rounded terminal valve of chain. a. Long, tapering spines from valve; b. Shorter spines close to valve; c. Orientation view. Valve not entirely clean between setae over center part of valve, but showing constriction on mantle and spines on setae. Scale: 10 $\mu$ m. 44: Rounded intercalary valve. a. Orientation view of valve broken from sibling pair with broken «prehensors» pointing back to the valve. Scale: 10 $\mu$ m; b. Detail of round, tube-formed labiate like process nestled under the close curving of the setae. Note pores in valve structure. 45: MD08, St. 5. a. Orientation view of sibling valves, one rounded and the other

rectangular in girdle view, both with labiate-like processes, and held together with «prehensor». Scale: 10 $\mu$ m, b. Detail of «prehensor» on seta from rounded valve holding sibling valves together.

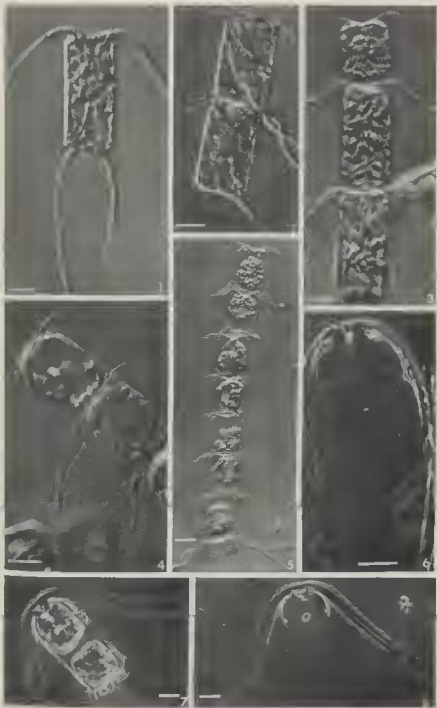
Fig. 46-49. *Chaetoceros convolutum* Castracane, British Columbia. Siliceous parts of diatom frustules cleaned of organic material SEM. Scale: 1 $\mu$ m, except where noted. 46: a. Rounded valve with parts of broken rectangular valve still attached. b. Detail of partially broken or worn «prehensor» from seta of rounded valve. c. Portion of seta with rectangular cross section, spines on angles, and double rows of poroids between ribs. 47: Another view of «prehensor» on seta. 48: Portion of broken seta with «prehensor» and double rows of poroids. b. Same seta. Note spines far from valve on reinforced angles of valve. 49: a. Sibling intercalary pair of valves looking into top valve and showing typical angle of setae. Scale: 10 $\mu$ m. b. Internal stellate pore of labiate-like process.

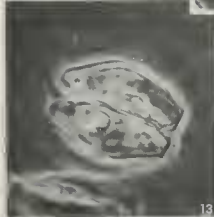
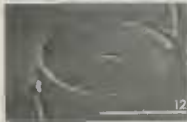
Fig. 50-52. — 50: *Chaetoceros flexuosum* Mangin, after Mangin, 1915. Broad girdle view. 51: *Chaetoceros radicans* Schütt, after Schütt, 1895. Valve view of sibling valves. 52: *Chaetoceros convolutum* Castracane, after Castracane, 1886. Broad girdle view and valve view of sibling valves.

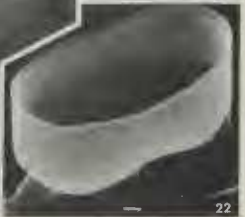
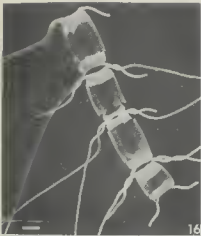
Fig. 53-54. — 53: *Chaetoceros criophilum* Castracane, after Mangin, 1917a. Broad girdle view. 54: *Chaetoceros concavicornis* Mangin, after Mangin, 1917b. Broad girdle view.

Fig. 55. — Resting spore formation of *Chaetoceros didymum* Ehrenberg adapted from von Stosch, Theil, and Kowallik, 1973. Sequence similar to *C. radicans*. A: Vegetative theca. B: Equal mitotic division forming sibling valves with specialized setae (SS). C: First heavily silicified arched resting (AR) spore valve formed inside original vegetative epitheca (VE). D: Second spore arched resting (AR) valve formed and first flattened resting (FR) spore hypovalve formed against valve with specialized setae. One vegetative epitheca (VE) still in place; the other lost. E: Two resting spores in typical conformation, held together by valves with specialized setae. F: Two resting spores (RS) dehiscing from valves with specialized setae (SS).



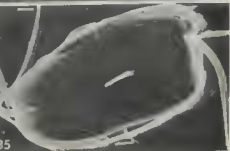
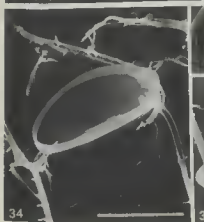
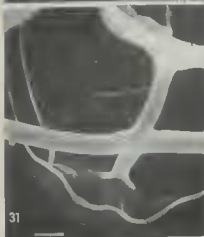
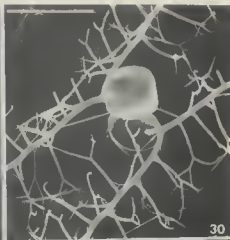


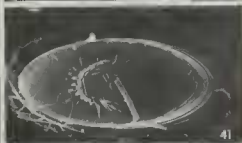
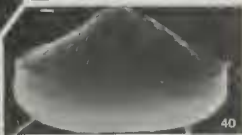
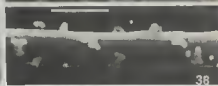
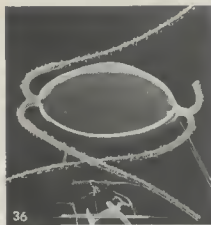


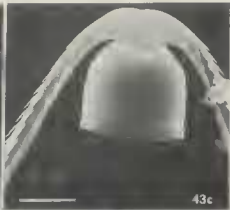


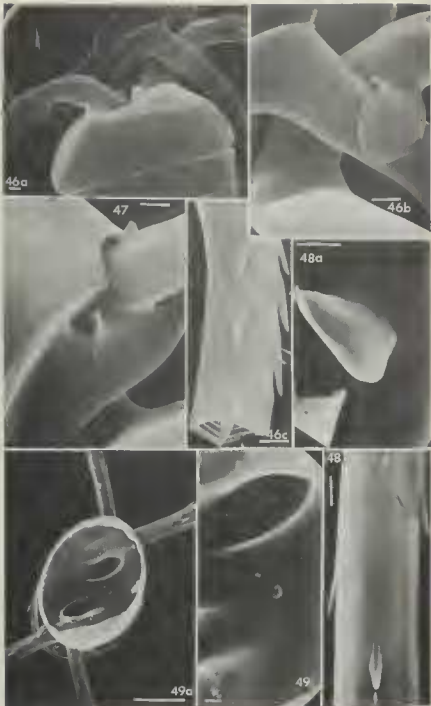
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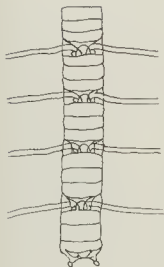


FIG. 52

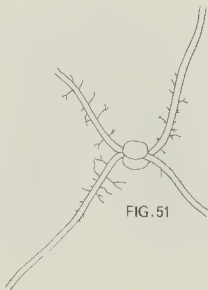
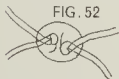


FIG. 51

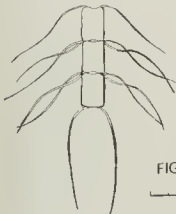
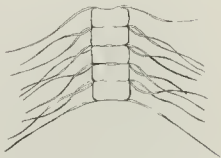
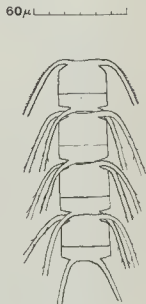
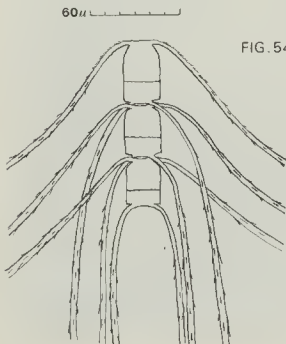
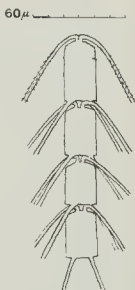
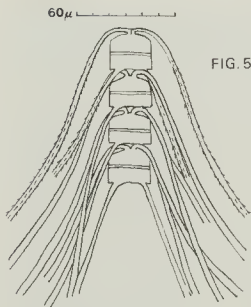


FIG. 50



60  $\mu$



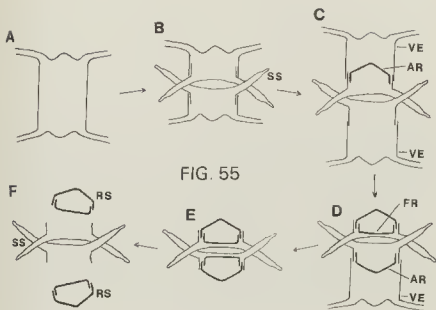


FIG. 55